

Correspondence

Nutrient enrichment and coral reproduction: empty vessels make the most sound (response to a critique by B. Rinkevich)**1. Background***1.1. The debate*

In a recent review, Loya (2004) provided a detailed account of changes that had taken place over more than three decades in the coral community structure of Eilat's coral reefs (northern Gulf of Aqaba/Eilat, GOA). This review provides an account of the major sources of the adverse anthropogenic effects on these reefs, ranging from physical damage due to increased human usage, to physiological damage resulting from coastal derived pollution, and in the past 10 years, an increase in eutrophication due to in situ net pen fish farming activity. There is no debate that, due to strong anthropogenic impacts, the coral reefs of Eilat, one of the most intensely studied reefs in the world, are undergoing a recently accelerated and worrying decline. However, there is disagreement as to the specific major causes of this decline (Bongiorni et al., 2003a; Loya et al., 2004).

Intensive net-pen fish farms situated in the northernmost section of the Gulf produce annually ca. 2500t, mostly of the sea bream *Sparus aurata*. These farms release ca. $18 \times 10^6 \text{ mol N yr}^{-1}$ and are the largest anthropogenic source of N and P into the northern Gulf (Atkinson et al., 2001).

Our experiment (Loya et al., 2004) constituted a pilot of a multi-tiered project aimed at ascertaining possible effects of Fish Cage (FC) effluents on the reef ecosystem in the GOA. Although this experiment is part of a long-term project, in which we are monitoring corals growing naturally and transplanted to a number of sites in the Northern Gulf, we saw fit to report the first set of results obtained, following 2 years of monitoring (2001–2002) growth and reproduction of the coral *Stylophora pistillata* at the FC site and a reference site (IUI). We believe that *two full reproductive seasons* (misleadingly portrayed by Rinkevich (2004) [RINK] as “a short (15 month) field experiment”), though not optimal, are

sufficient to ascertain reproductive trends in the relatively short lived r-strategist coral *S. pistillata* (Loya, 1976).

Monitoring a *single coral reproductive season*, Rinkevich's group (Bongiorni et al., 2003a [BEA]) concluded that the reproductive activities (i.e., oocyte numbers and size distribution) of *S. pistillata* were higher at the fish farm site than at the IUI reference site. In contrast, our study of the same coral species, transplanted to the same sites, carried out during two reproductive seasons (which were in part, in parallel with BEA's study), and examining a larger number of reproduction parameters, concluded that the fish cages (FC) adversely affect reproduction in this coral (Loya et al., 2004). Moreover, we claimed that BEA's findings not only suggest the opposite of their conclusions (see Loya and Kramarsky-Winter, 2003) but in fact reinforce our own results and conclusions.

1.2. 'The camel never sees its own hump'

RINK “criticized” our methods, results and conclusions. Regrettably, in his “great effort” to uncover “the truth” and in his haste to find in our paper “self-contradictions”, “discrepancies”, “inaccuracies”, “negligence to genuinely analyze results”, intentional “data omissions and elimination of results”, etc., etc., RINK fails to see the absurdity of his own accusations. Our response reveals RINK's neglect to read carefully and/or perhaps comprehend both the body of the text and the Figure and Table legends in our paper.

1.3. Headings and style

Although it is not our usual style of writing, we adopt, in the “spirit of science”, the heading style chosen by RINK (see also Rinkevich et al., 2003 title). In the following, we use “double quotes” in quoting or referring to RINK and ‘single quotes’ in quoting or emphasizing our own text. For clarity's sake, we retain the same headings and order of RINK's claims, followed by heading(s), portraying our view on the claim.

2. Claim: “Erroneous experimental design”.

Reply: ‘Much ado about nothing’

RINK lists “four major flaws” in our experimental design which “invalidate” our conclusions that, “otherwise, could have been drawn from the results”:

2.1. “Choice of site”

The makeup of the sandy substrate near the FC is indeed different from the coarser sand at the IUI, but this does not change the fact that at both sites colonies of the ‘weedy’ *S. pistillata* are found growing naturally on submerged objects, and some colonies are even found growing naturally on the FC substrate. Moreover, our transplanted colonies were not placed “a few centimeters above the sand” as misleadingly pointed out by RINK, but rather at a height of 50–75cm above the bottom ‘attached to tiles secured to the underside of plastic crates by underwater putty’. The source of RINK’s misinformation is unclear. In our opinion, transplanting the corals close to but sufficiently above the sea bottom substrate (thus avoiding “suffocation” of the transplanted corals by possible re-suspension of sediments) is ecologically more relevant than placing them in mid-water (e.g., 6m depth), as done by BEA for a number of reasons: (1) Corals do not usually grow naturally in mid-water (the depth in the FC site is ca. 20m). Colonies that may have been placed in mid-water far from the effects of sediment (as in BEA’s experiment) would not reflect “natural conditions”, as they may be flushed by water on all surfaces, keeping them clean of particulate matter and not taking into account natural factors, such as the effect of substances adsorbed to and released from the sediment. (2) If our transplanted corals had been placed in shallow waters (e.g., as in BEA) they would have been subjected to only a portion of the effluent emanating from the FC, since a large proportion of the fish in the net pens are located deeper than 6m. Particulate matter released from the FCs may drop to the sediment and thus not reach shallow water colonies. (3) Rinkevich’s group (Bongiorni et al., 2003b) found that even when grown in mid-water not all fragments in their FC site fared well. Growth rates increased only in those fragments that were placed perpendicular to the water surface, indicating that particulate matter had an effect on the corals. Throughout their experiment, BEA removed from their settling plates “algae and encrusting invertebrates on a monthly basis”, while we refrained from interference with any environmental parameter. In doing so, BEA falsely eliminated one of the most important environmental effects of fish farms on corals: i.e. stimulating growth of benthic algae that may smother and competitively exclude the corals (see Loya and Kramarsky-Winter, 2003).

2.2. “Choice of coral placement”

Throughout his critique, RINK struggles to expose “inaccuracies” in our publications. Stating that “none” of our results can be attributed to chronic fish cage effluents, he claims that we reported in one publication to have placed our transplanted corals 150m west of the Fish Cages (Loya et al., 2004), while pointing out an “inaccuracy” in another publication concerning the above distance, i.e., “unjustly claimed, by the same authors, to be 200m from the fish cages”. In our papers there is indeed an “inaccuracy” due to our visually-based estimate of the distance between our experimental site and the fish cages and we thank RINK for pointing this out. After actual measurement of the above disputed distance, we found it to be 70m west of the westernmost net-pen and approximately 100m west of the easternmost net-pen, i.e., even closer than our previously reported approximation, thus, even strengthening our conclusions.

RINK’s assertion that levels of nutrients drop to background levels at a distance of less than 150m is an oversimplification. This assertion may have resulted from the erroneous use of data in the reports cited by RINK, since nutrient measurements in these reports were carried out at 2m depth. High primary production occurring in the top water layers and wind-driven surface water currents in that area would, as expected, result in low nutrient levels, in shallow water levels at short distances from the FC. However, recent periodic samples taken during stratification showed higher nutrient levels at our FC site (19m depth) when compared to the same depth at the IUI site (G. Winters, pers. comm.).

2.3. “Choice of coral source and proper controls”

RINK states that we “neglected to carefully plan the experiment and to genuinely analyze the results”. Our collection site was at ca. 10–15m depth, outside the port of Eilat, far indeed from being a ‘pristine reef’ and in conjunction with restrictions and permits received from the Israel Nature Conservation Authority. However, the fact that the collected colonies were transplanted to both the FC site and reference site (IUI), negates the claim that there was “bias” in our experiment. Obviously, such corals were suffering stress, incurred through their removal from their natural surroundings and subsequent transplantation. Nevertheless, the colonies underwent the same primary treatment and acclimatized for 5 months prior to first sampling. Hence, we could expect the major difference in reproductive effort to be a result of the site they were transplanted to.

The use of resident (native) colonies would certainly have been a better parameter for ascertaining the effect of fish farms on natural populations. This is true had the colonies that had grown at the FC site been monitored prior to deployment of the cages, and the subsequent effect of the FC been studied. This unfortunately was not carried out, but should indeed have been done prior to and during the early phase of deployment of the cages, in order to understand the effects they may have had on the environment. Regrettably, we were not informed nor asked to give opinions on the impending deployment of the cages during the “environmental assessment phase” that the mariculture companies undertook, and no study was conducted as to the possible effects of the FC on coral physiology. Furthermore, no environmental assessment was carried out prior to the impending exponential increase in fish cage numbers and fish yield (ca. 1995) that the fish companies undertook. Thus, such a comparison could not have been carried out.

It is possible that had we decided to study potential effects of the FC's on the reproductive effort of resident populations of *S. pistillata*, we might have found different results. We claim, however, that this is unlikely, since we obtained comparable results to those reported by BEA, when comparing results where similar parameters and methodologies were used, i.e. (1) The percentage of polyps with testes, both from resident populations studied by BEA and transplanted populations (our study) in the same study sites were similar. (2) The trends found in lipid contents of the transplanted colonies from our study were comparable to those reported by BEA in resident populations of *S. pistillata* at the same two sites. Unfortunately we could not compare other parameters due to differences in methodologies.

2.4. “Choice of procedures for histological sections and lipid extraction”

RINK “invalidates” our methodology of lipid studies, which corroborated our assertion of significantly greater planulae production by IUI colonies than FC colonies. Again, this “nullification” is done by providing a misleading interpretation of our text. Our sampling procedure for lipid content entailed a process where each branch was divided into two parts: the bottom part was used to determine reproductive effort, while the ‘upper part’ i.e., top half (3–4 cm) was used for lipid extraction. Although the very top 1 cm is usually devoid of gonads, the rest of the tissue contains gonads. Indeed, exactly the same methodology was used by Rinkevich (see BEA). Moreover and most importantly, the lipid levels found in our study were comparable with those reported in BEA; it is our interpretations and conclusions that conflict with theirs.

3. Claim: “Incongruity between text and figures”.

Reply: ‘Empty vessels make the most sound’

3.1. ‘About truths, half-truths and false assertions’

It is regrettable that assertions such as “However their results were based on only five to nine colonies,” are not even ‘half-truths’. In fact, such statements are both false and misleading. This may have been a result of careless reading on RINK's part and/or on his failure to comprehend the text of Table 1. The correct sample sizes, as clearly indicated in Table 1 are, $n = 10$ colonies for each one of the years 2001 and 2002 in the FC site, and $n = 12$ and 14 for the years 2001 and 2002, respectively. At each site, a minimum of 10 colonies was studied per year (e.g., five colonies at the FC site during March 2001 and then five *different* colonies during May 2001). Similarly, at the IUI site six to nine *different* colonies were sampled during each of the two sampling periods in 2001 and 2002. We emphasized in Table 1 that ‘each set of colonies was sampled only once a year in order to prevent re-sampling of a colony that had been injured by the sampling procedure’.

In attempting to expose “contradictions”, “inaccuracies”, and “discrepancies” in our data, RINK misleadingly uses partial quotes from our paper, claiming, e.g., that we examined in histological sections “50–60 polyps” per colony, whereas in our paper we clearly stated that the sections ‘each containing approximately 50–60 polyps were examined histologically’. The total number of polyps actually counted for ascertaining number of gonads was lower than the sum of the polyps in each section, because we only examined those that were whole in the section. This “discrepancy” may have resulted from our precision in reliably providing the exact number of polyps examined in every sampling period at both sites. Hence, RINK's unacceptable implications of intentional “data omissions”, “elimination” of results, failure to “genuinely analyze” results, etc., approach absurdity.

3.2. ‘On the difference between standard error (SE) and standard deviation (SD)’

The “discrepancies” and “contradictions” that RINK tries so hard to read into our results, (“what then are the real figures?”) are sheer nonsense. Had he read the text of Fig. 2, he would have realized that the graphs were presented using standard errors (SEs). This was in order to be able to compare our results with those reported by BEA, who chose to present SEs in their graphs. Furthermore, in the text to our Materials and Methods it was stated that ‘results are presented as \pm standard deviations unless denoted otherwise’ (as indeed indicated in the legend to our Fig. 2).

3.3. 'On some basic knowledge of coral reproduction biology'

In “judging” our data, RINK misleads the reader in asserting that “it is implausible that any of the oocytes in the histological sections could actually reach the mature size”. Sadly, he seems to have “forgotten” some basic knowledge that he himself contributed to the field concerning the reproduction biology of *S. pistillata* (Rinkevich and Loya, 1979). The decrease in average oocyte sizes of *S. pistillata* occurring between March (mid reproductive period) and May (toward the end of the reproductive period) occurred at the IUI site, as more and more oocytes were fertilized and developed into planulae. No such decrease in oocyte numbers occurred at the FC site, presumably due to the fact that only a few of the oocytes were fertilized. For detailed discussion on this subject see Discussion in Loya et al. (2004).

It is puzzling why RINK chose to ignore the most significant parameter for understanding *reproductive effort* in this species i.e., the number of planulae produced by each colony. Inevitably, it is the number of offspring that each colony produces that reflects its reproductive effort. The most important and unequivocal result presented in our paper was the significantly higher planulae numbers found in corals transplanted to the IUI reference site, when compared with those transplanted to the FC site. RINK excuses himself from matching this weighty result by picking on our legend to Fig. 4; i.e., using the term “annual reproductive period, a legend that may lead readers to a fallacious conclusion”. A careful reading of the text referring to Fig. 4 in our paper clearly refutes this marginal claim. Overlooking this parameter is particularly surprising, since Rinkevich himself used this very parameter to ascertain reproductive fitness of this coral species in previous publications (Rinkevich and Loya, 1989). Nevertheless, and perhaps not surprisingly, he failed to do so in his recent publication concerning the reproduction of *S. pistillata* in the vicinity of the fish farms (see BEA). Hence, we were rather amused by RINK's accusation that we “omitted” from our data “basic reproductive parameters of corals, such as number of oocytes per polyp”.

4. Claim: “What do the results portray?”. Reply: “One cannot see the wood for the trees”

Although our study recorded *two* consecutive reproductive seasons, we were only able to compare ‘reproductive effort’ of our transplanted colonies to that of resident colonies studied by BEA, in a *single*, parallel season to ours (2001). The results presented in our Table 2 clearly indicate the high similarity in the percentage of colonies containing oocytes in both BEA and our studies at the IUI site, but *not* at the FC site. Possible rea-

sons for this were discussed in the paper. We invite the readers to judge for themselves (i.e., compare BEA to Loya et al., 2004) concerning reproductive parameters studied in both studies and, in particular, see the discussion on ‘reproductive effort’.

RINK provides lengthy and rather ambiguous calculations, concerning percentages of polyps containing male gonads, female gonads etc., as well as misleading statements such as, “sterile colonies were characterized in Table 1 as being hermaphrodites!”. Unfortunately, ‘one cannot see the wood for the trees’ in RINK's “criticism”. We urge the reader to examine our original figures and text, which clearly present the details confused by RINK. It is probable that careless reading of the text and hasty assessment of the Figures and Tables in our paper resulted in RINK's “astonishment” that hermaphrodite corals contained no oocytes. What he failed to notice however was that they did contain planulae. Thus, in June 2002 none of the colonies at the IUI had oocytes (see Fig. 3b in Loya et al., 2004), and they only contained very few testes (Fig. 2), but they *did* contain planulae (Fig. 3c). It is our understanding that colonies containing planulae and testes are by definition hermaphrodites. Thus, RINK's accusation of our “negligence to genuinely analyze” our results is puzzling if not enigmatic, to say the least. Regrettably, his “unveiling” of “self-contradictions” between the Figures and Tables in our paper merely illustrates slipshod reading.

Certainly, when compared with previous studies, reproductive effort in *S. pistillata* in the northern GOA has been severely reduced. This reduction was corroborated in a recent but as yet unpublished study (Zakai, pers. comm.) that showed a reduction in fecundity and reproductive effort of resident *S. pistillata* colonies at the IUI over recent years. This is unfortunately one of the signals of a highly stressed reef and perhaps indicates a reef-wide degradation. However, it does not change the fact that the reproductive effort in colonies from the FC site was still significantly lower than in colonies at the IUI.

The effect of nutrification on coral reproduction will only be unequivocally proven by carefully controlled laboratory experiments (as was shown by Cox and Ward, 2003). Our study, on the other hand, was an *in situ* experiment that showed that the transfer of colonies close to the FC significantly depressed reproductive effort of this species, compared with reproductive effort of colonies transferred from the same origin to a more “pristine” site—the IUI. It is of course likely that no one factor is solely responsible for this depressed reproductive effort. This state may be a result of synergistic effects of elevated nutrients, sedimentation and particulate matter, as well as the presence of unwanted chemicals such as antifouling substances, hormones and antibiotics. Despite the lack of direct evidence as to

the causative agent(s), it is clear that the colonies that had been transferred to the FC site were faring worse than those transplanted to the IUI.

5. Claim: “Between truth and repose”. Reply: ‘Vincit omnia veritas’ (L)–‘Truth conquers all’

RINK preaches to establish facts and expose “the truth” on the basis of data published in refereed journals only (see Rinkevich et al., 2003). It is still unclear why he chose to ignore recent publications discussing the role of the fish farms in the deterioration of Eilat’s reefs (Abelson et al., 1999; Ben-Tzvi et al., 2004; Loya, 2004; Loya et al., 2004; Silverman et al., 2004). His selective choice of references has led him to state that “continuous deterioration of Eilat’s reef is a direct result of effluents released from the fish farms . . . is not scientifically documented”. To corroborate this conclusion, however, he is able to quote only his own studies (criticized by Loya and Kramarsky-Winter, 2003) and that of Golani and Lerner (2003), an abstract presented in a local conference.

It is disappointing that RINK saw fit to doubt and attack the validity of our results without providing any actual data that could refute or disagree with them. This is particularly disturbing in view of the fact that our results (Loya et al., 2004) are comparable with the results published by BEA. Our disagreements arise only in the interpretations and conclusions they presented. Does this mean that Rinkevich does not believe in his own results too? Despite his “desire” for “the truth” to be exposed, he neglects to relate to the most *important and critical* finding in our research: i.e., the fact that colonies transplanted to the polluted FC site produced significantly fewer planulae than those transplanted to the IUI reference site.

Regrettably, this debate has recently gone beyond scientific discussion and developed into a personal and economically driven dispute. We can only plead that scientific research and discussion remain just that, and do not become motivated by economic or personal incentives. When that happens, scientific research becomes biased and is by definition no longer scientific. In the present FC dispute, we leave it to the readers to judge as to ‘who is fair and who is foul?’ (sensu Rinkevich et al., 2003).

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